UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

June 29, 2007

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 74986-4, Selective Micro Clean-Alpha:
DP Barcode: 338835

From: Tajah L. Blackburn, Ph.D., Microbiologist
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510P)

6/29/07

Thru: Michele Wingfield, Chief
Product Science Branch
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To: Emily Mitchell PM 32/ Wanda Henson
Regulatory Management Branch II
Antimicrobials Division (7510P)

Applicant: Selective Micro Technologies
5 Cherry Hill Drive
Danvers, MA 01923

Formulations from Label

Active Ingredient(s)	% by wt.
Sodium Chlorite.....	30.5%
Other Ingredients.....	69.5%
Total	100.0%

I BACKGROUND

The product, Selective Micro Clean-Alpha (EPA Reg. No. 74986-4), is a registered disinfectant (bactericide, fungicide, tuberculocide, virucide), sanitizing rinse (food contact), sanitizer (non-food contact), algaecide, slimicide, and deodorizer for use on hard, non-porous surfaces in commercial, industrial, institutional, food preparation, food processing, animal care, and hospital or medical environments. The product consists of a 0.44 ounce pouch containing sodium chlorite as the active ingredient. With the addition of 2 liters of water, the product produces 500 ppm aqueous chlorine dioxide within 6 hours. This activated solution is then diluted dependent on the application. The applicant requested to amend the product registration (1) to add claims for effectiveness as a disinfectant against *Listeria monocytogenes*, *Candida albicans*, Adenovirus, Canine parvovirus, Herpes simplex type 2, Influenza A virus, Norovirus, Rhinovirus, and Vaccinia virus; (2) to add claims for effectiveness as a sanitizer against *Listeria monocytogenes*; (3) to replace the primary brand name, Selective Micro Clean-Alpha, with the name, Selectrocide 2L500; (4) to modify language labels and technical bulletins; and (5) to add instructions for wineries and additional use patterns (non-public health pathogens). Studies were conducted at MicroBioTest, Inc., located at 105 Carpenter Drive, in Sterling, VA 20164; and ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative to EPA (dated March 22, 2007), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-35 (Data Matrix), twelve studies (MRID 470896-03 through 470896-14), Statements of No Data Confidentiality Claims for all twelve studies, the last accepted label (dated December 14, 2005), the proposed label, and a revised technical bulletin for the product.

II USE DIRECTIONS

The proposed label provided the following instructions for activating the product: Fill the pouch with 2 liters of tap water. Wait at least 6 hours before use to ensure that the solution reaches full strength. Shake gently before use. Before use, verify the concentration using *Selective Micro Chlorine Dioxide Test Strips*. Activate the product prior to the expiration date stamped on the pouch. Use the activated solution within 15 days of activation.

The product is designed to be used for disinfecting hard, non-porous surfaces. The Technical Bulletin identifies such surfaces to include: bench tops, biological hoods, ceilings, equipment, floors, incubators, instruments, kennels, stainless steel cold rooms, tanks, transfer lines, walk-in incubators, and walls. Directions on the proposed label provided the following information regarding preparation and use of the product as a disinfectant: Prepare a 100 ppm use solution by diluting 1 part 500 ppm solution to 4 parts water. Apply the use solution using a mop, sponge, or sprayer, or by immersion. Surfaces must remain wet for 10 minutes.

The product is also designed for sanitizing hard, non-porous, non-food contact surfaces such as equipment, floors, furnishings, and walls. Directions on the proposed label provided the following information regarding preparation and use of the product as a sanitizer: Prepare a 20 ppm use solution by diluting 1 part 500 ppm solution to 24

parts water. Apply the use solution using a mop, sponge, or sprayer, or by immersion. Surfaces must remain wet for 5 minutes.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are presented in DIS/TSS-1.

Disinfectants for Use as Fungicides (Using a Modified AOAC Use-Dilution Method)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required. These Agency standards are presented in DIS/TSS-6.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes, Aspergillus niger,* and *Candida albicans.* EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable,

results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

Sanitizers (For Non-Food Contact Surfaces)

There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is to be used as a sanitizer for non-food contact surfaces. Confirmatory test standards would apply. Therefore, 2 product samples, representing 2 different product lots, should be tested against each additional microorganism. Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

IV SYNOPSIS OF SUBMITTED EFFICACY STUDIES

1. MRID 470896-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus type 37," for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 6, 2006. Project Number A03515.

This study was conducted against Rhinovirus type 37 (Strain 151-1; ATCC VR-1147) using MRC-5 cells (human embryonic lung cells; ATCC CCL-171; propagated in-house) as the host system. Two lots (Lot Nos. W05298-01 and W05300-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01091305.RHV (copy requested). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 242 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~19.5 hours at 20.0°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 255 ppm). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C in 52% relative humidity. For each product lot, one dried virus film was treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and

neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

2. MRID 470896-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Canine Parvovirus," by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 6, 2006. Project Number A03516.

This study was conducted against Canine parvovirus (Strain Cornell; ATCC VR-2017) using A-72 cells (canine tumor cells; ATCC CRL-1542; propagated in-house) as the host system. Two lots (Lot Nos. W05298-01 and W05300-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01091305.CPV (copy not provided, but requested). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 242 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~41 hours at 20.0°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 255 ppm). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C in 60% relative humidity. For each product lot, one dried virus film was treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. A-72 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 14 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. On the final day of incubation, a hemagglutination assay was performed on the cultures using swine red blood cells at 2-8°C. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

Note: The protocol is amended to change the incubation time listed in the infectivity assay section of the protocol from approximately seven days to seven to fourteen days.

The change in incubation time is due to the unavailability of the swine red blood cells needed to perform the hemagglutination assay on the final day of the incubation.

3. MRID 470896-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 5," for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 6, 2006. Project Number A03517.

This study was conducted against Adenovirus type 5 (Strain Adenoid 75; ATCC VR-5) using A-549 cells (human lung carcinoma; ATCC CCL-185; propagated in-house) as the host system. Two lots (Lot Nos. W05298-01 and W05300-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01091305.ADV (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 242 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~41 hours at 20.0°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 255 ppm). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C in 60% relative humidity. For each product lot, one dried virus film was treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. A-549 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

4. MRID 470896-06 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 2," for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 6, 2006. Project Number A03518.

This study was conducted against Herpes simplex virus type 2 (Strain G; ATCC VR-734) using rabbit kidney cells (obtained from ViroMed Laboratories, Inc.; maintained in-house) as the host system. Two lots (Lot Nos. W05298-01 and W05300-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No.

SMT01091305.HSV2 (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 242 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~19.5 hours at 20.0°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 255 ppm). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C in 51% relative humidity. For each product lot, one dried virus film was treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. Rabbit kidney cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

5. MRID 470896-07 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus," for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 6, 2006. Project Number A03519.

This study was conducted against Influenza A virus (Strain Hong Kong; ATCC VR-544) using Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Inc.; maintained in-house) as the host system. Two lots (Lot Nos. W05298-01 and W05300-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01091305.FLUA (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 242 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~41 hours at 20.0°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 255 ppm). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C in 53% relative humidity. For each product lot, one dried virus film

was treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

6. MRID 470896-08 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Vaccinia virus," for Selective Micro Clean-Alpha, by Karen M. Ramm. Study conducted at ATS Labs. Study completion date – February 6, 2006. Project Number A03520.

This study was conducted against Vaccinia virus (Strain WR; ATCC VR-119) using Vero cells (ATCC CCL-81; propagated in-house) as the host system. Two lots (Lot Nos. W05298-01 and W05300-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Labs Protocol No. SMT01091305.VAC (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 242 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~19.5 hours at 20.0°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 255 ppm). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C in 52% relative humidity. For each product lot, one dried virus film was treated with 2.0 ml of the use solution for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

7. MRID 470896-09 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus," for Selective Micro Clean-Alpha, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – April 27, 2006. Project Number A03799.

This study, under the direction of Study Director Kelleen Gutzmann, was conducted against Feline calicivirus (F-9 strain; ATCC VR-782) using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. W06026-01 and W06030-01) of the product, Selective Micro Clean-Alpha, were tested according to ATS Lab Protocol No. SMT01030106.FCAL.2 (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 254 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~24 hours at 18.5°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 250 ppm). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 18.5°C at 23% relative humidity. For each lot of product, two separate dried virus films were treated with 2.0 ml of the use solution for 10 minutes at 18.5°C. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus titer, dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The $\log_{10}$ reduction in infectivity was also calculated using the revised EPA approved method for calculating the Most Probable Number (MPN).

8. MRID 470896-10 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus – Confirmatory Assay," for Selective Micro Clean-Alpha, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – April 20, 2006. Project Number A03800.

This study, under the direction of Study Director Mary J. Miller, was conducted against Feline calicivirus (F-9 strain; ATCC VR-782) using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. One lot (Lot No. W06026-01) of the product, Selective Micro Clean-Alpha, was tested according to ATS Lab Protocol No. SMT01030106.FCAL.1 (copy not provided). A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250 ppm AOAC synthetic hard water (titrated at 259 ppm) to the sponsor-provided product (presumably the contents of a pouch of dry ingredients). The use solution was held for ~24 hours at 18.5°C. Following activation, the use solution was thoroughly mixed by inversion and the chlorine dioxide concentration was determined. A 100 ppm use solution was then prepared by diluting the activated solution with 250 ppm AOAC synthetic hard water (titrated at 250 ppm). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 ml of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 19.0°C at 24% relative humidity. For the single product lot, two separate dried virus films were treated with 2.0 ml of the use solution for 10 minutes at 19.0°C. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/ml gentamicin, 100 units/ml penicillin, and 2.5 µg/ml amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 ml of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus titer, dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The $\log_{10}$ reduction in infectivity was also calculated using the revised EPA approved method for calculating the Most Probable Number (MPN).

9. MRID 470896-11 "Use Dilution Test – Supplemental *Listeria monocytogenes*," for Selective Micro Clean-Alpha, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – December 23, 2005. Laboratory Project Identification Number 478-157.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 5215-01 and 5216-01) of the product, Selective Micro Clean-Alpha, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250±2.9% ppm AOAC synthetic hard water to the sponsor-provided product. The use solution was held for at least 6 hours at room temperature. A 100 ppm use solution was then prepared by diluting the activated solution with 250±2.9% ppm AOAC synthetic hard water. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 20

carriers per 20 ml broth. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was exposed to 10 ml of the use solution for 10 minutes at 20°C. Following exposure, the carriers were transferred to Brain Heart Infusion Broth with 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for dried carrier counts, sterility, viability, bacteriostasis, neutralizer effectiveness, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: Hach Chlorine Dioxide Analysis Test Strip method and Direct Reading method were used to determine available chlorine.

Note: The laboratory report stated that an assay initiated on September 19, 2005 resulted in an invalid assay. No other information about this assay was provided.

10. MRID 470896-12 "Use Dilution Test – Supplemental *Candida albicans*," for Selective Micro Clean-Alpha, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – December 23, 2005. Laboratory Project Identification Number 478-161.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos. 5215-01 and 5216-01) of the product, Selective Micro Clean-Alpha, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250±2.9% ppm AOAC synthetic hard water to the sponsor-provided product. The use solution was held for at least 6 hours at room temperature. A 100 ppm use solution was then prepared by diluting the activated solution with 250±2.9% ppm AOAC synthetic hard water. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per 20 ml broth. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was exposed to 10 ml of the use solution for 10 minutes at 20°C. Following exposure, the carriers were transferred to Tryptic Soy Broth with 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for dried carrier counts, sterility, viability, fungistasis, neutralizer effectiveness, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

11. MRID 470896-13 "Use Dilution Test – Supplemental *Candida albicans*," for Selective Micro Clean-Alpha, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – December 23, 2005. Laboratory Project Identification Number 478-162.

This study was conducted against *Candida albicans* (ATCC 10231). Two lots (Lot Nos. 5215-01 and 5216-01) of the product, Selective Micro Clean-Alpha, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250±2.9% ppm AOAC synthetic hard water to the sponsor-provided product. The use solution was held for at least 6 hours at room temperature. A 50 ppm use solution was then prepared by diluting the activated solution with 250±2.9% ppm AOAC synthetic hard water. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 20 carriers per 20 ml broth. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was exposed to 10 ml of the use solution for 20 minutes at 20°C. Following exposure, the carriers were transferred to Tryptic Soy Broth with 0.1% sodium thiosulfate to neutralize. All subcultures were incubated for 48±2 hours at 37±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for dried carrier counts, sterility, viability, fungistasis, neutralizer effectiveness, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

12. MRID 470896-14 "Sanitizer Test for Non-Food Contact Surfaces," Test Organism: *Listeria monocytogenes* (ATCC 19111), for Selective Micro Clean-Alpha, by Angela L. Hollingsworth. Study conducted at MicroBioTest, Inc. Study completion date – December 23, 2005. Laboratory Project Identification Number 478-156.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). Two lots (Lot Nos. 5215-01 and 5216-01) of the product, Selective Micro Clean-Alpha, were tested. The laboratory study referenced the Sanitizer Test from DIS/TSS-10. A >500 ppm ClO_2 use solution was prepared by adding 2.0 L of 250±2.9% ppm AOAC synthetic hard water to the sponsor-provided product. The use solution was held for at least 6 hours at room temperature. A 20 ppm use solution was then prepared by diluting the activated solution with 250±2.9% ppm AOAC synthetic hard water. Heat-inactivated horse serum was added to the culture to achieve a 5% organic soil load. Three sterile, glass square carriers per product lot were inoculated with 0.01-0.03 ml of an 18-24 hour old suspension of the test organism. The carriers were dried for 20-40 minutes at 37±2°C. Each carrier was exposed to 5.0 ml of the use solution for 5 minutes at 21°C. After exposure, excess liquid was allowed to drain off and each carrier was transferred to a 32 x 200 mm tube containing 20 ml of stripping fluid (i.e., 0.85% NaCl with 0.1% Triton X-100) with 0.1 sodium thiosulfate. The tubes were subjected to ultrasound for 5 minutes in a cleaning sonicator. The test suspensions were serially diluted tenfold in phosphate buffer saline dilution blanks. Duplicate aliquots of selected dilutions were plated in Brain Heart Infusion Agar plates. All plates were incubated for 48±2 hours at 37±2°C. Following incubation, the colonies were counted. Controls included those for

zero-time counts, parallel counts, neutralizer effectiveness, sterility, and confirmation of the challenge microorganism.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable, as documented.

Note: The laboratory report stated that an assay initiated on September 19, 2005 resulted in an invalid assay. No other information about this assay was provided.

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Dried Carrier Counts (CFU/carrier)
		Lot No. 5215-01	Lot No. 5216-01	
470896-11	*Listeria monocytogenes*	0/10	0/10	2.1×10^6
470896-12	*Candida albicans*	0/10	0/10	1.9×10^5
470896-13	*Candida albicans*	0/10	0/1	1.9×10^5

MRID Number	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 mL)
			Lot No. W05298-01	Lot No. W05300-01	
470896-03	Rhinovirus type 37	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
470896-04	Canine parvovirus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
470896-05	Adenovirus type 5	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
470896-06	Herpes simplex virus type 2	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$
		$TCID_{50}$/1.0 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
470896-07	Influenza A virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.0}$
		$TCID_{50}$/1.0 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
470896-08	Vaccinia virus	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
			W06026-01	W0603-01	

MRID Number	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 mL)
470896-09	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{6.0}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
470896-10	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	$10^{6.5}$ and $10^{6.0}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	---	

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/mL)		
470896-14	*Listeria monocytogenes*	5215-01	$<1.0 \times 10^1$	1.4×10^4	>99.9
		5216-01	$<1.0 \times 10^1$	1.4×10^4	>99.9

VI CONCLUSIONS

1. The submitted efficacy data support the use of the product, Selective Micro Clean-Alpha (aka Selectrocide 2L500), as a disinfectant on hard, non-porous surfaces against the following microorganisms in the presence of 250 ppm hard water and a 5% organic soil load under the following conditions:

Listeria monocytogenes	100 ppm	10 minutes	MRID 470896-11
Candida albicans	100 ppm	10 minutes	MRID 470896-12
Candida albicans	50 ppm	20 minutes	MRID 470896-13

Complete killing was observed in the subcultures of all carriers tested against the required number of product lots. Dried carrier counts for the *Listeria monocytogenes* study were at least 10^4 CFU/carrier. Dried carrier counts for the *Candida albicans* studies were at least 10^4, which is consistent with Agency's interim policy. [For more details about this interim policy, see Section III of this efficacy report.] Neutralizer effectiveness testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility and bacteriostasis/ fungistasis controls did not show growth.

2. The submitted efficacy data support the use of a 100 ppm use solution of the product, Selective Micro Clean-Alpha (aka Selectrocide 2L500), as a disinfectant with virucidal activity on hard, non-porous surfaces against the following microorganisms in the presence of 250 ppm hard water and a 5% organic soil load for a contact time of 10 minutes:

Adenovirus type 5	MRID 470896-05
Canine parvovirus	MRID 470896-04
Feline calicivirus (a surrogate for Norovirus)	MRID 470896-09 and -10
Herpes simplex virus type 2	MIRD 470896-06
Influenza A virus	MRID 470896-07
Rhinovirus type 37	MRID 470896-03

Recoverable virus titers of at least 10^4 were achieved. Complete inactivation (no growth) was indicated in all dilutions tested. Initial and confirmatory studies for Feline calicivirus were performed at the same laboratory but under different study directors. Confirmatory studies used one lot of product, not the standard two.

3. The submitted efficacy data support the use of a 20 ppm use solution of the product, Selective Micro Clean-Alpha (aka Selectrocide 2L500), as a non-food contact surface sanitizer on hard, non-porous surfaces against *Listeria monocytogenes* in the presence of 250 ppm hard water and a 5% organic soil load for a contact time of 5 minutes. At least a 99.9% reduction in population was observed in the subcultures of all carriers tested against the required number of product lots. Neutralizer effectiveness testing showed positive growth of the microorganism. Sterility controls did not show growth.

VII RECOMMENDATIONS

1. The proposed label claims that a 100 ppm use solution of the product, Selective Micro Clean-Alpha (aka Selectrocide 2L500), is an effective disinfectant for use on hard, non-porous surfaces against the following microorganisms for a contact time of 10 minutes:

Listeria monocytogenes
Candida albicans
Adenovirus
Canine parvovirus
Herpes simplex-2
Influenza A
Norovirus
Rhinovirus
Vaccinia

Data provided by the applicant support these claims. Claims for Norovirus should include language "Feline Calicivirus" as the tested organism, in some version.

2. The proposed label claims that a 50 ppm use solution of the product, Selective Micro Clean-Alpha (aka Selectrocide 2L500), is an effective disinfectant for clean-in-place applications for potable water systems against *Candida albicans* for a contact time of 20 minutes. Data provided by the applicant support this claim.

3. The proposed label claims that a 20 ppm use solution of the product, Selective Micro Clean-Alpha (aka Selectrocide 2L500), is an effective sanitizer for use on hard, non-porous, non-food contact surfaces against *Listeria monocytogenes* for a contact time of 5 minutes. Data provided by the applicant support this claim.

4. The newly added instructions for using the product in antimicrobial applications for water-based cutting fluids/ oils [see page 5 of the proposed label] differ from the newly added instructions provided on the label for the product, Selective Micro Clean 1G-1. The applicant may want to confirm that this difference is intentional.

5. ATCC designation numbers should be included tested organisms on the Data Matrix (EPA Form 8570-35), Technical Bulletin, or the proposed label.

6. Claims "to extend shelf-life and freshness of fruits and vegetables in food processing facilities" by the application of the product to fruits and vegetables are not acceptable. The Agency has not accepted any test protocols, nor developed a performance standard for this claim. Only claims for the use in "processing water" are acceptable.

7. On page 15 of the Technical Bulletin, include a contact time, representative of the test system, for "Sanitizing Final Rinse of Pre-Cleaned or New returnable or Non-returnable Containers."

8. The following changes are required on the proposed label:

- On page 3 of the proposed label, change "Rotavirus Influenza-A" to read "Rotavirus, Influenza A virus."

- On page 3 of the proposed label, change "Rhinovirus" to read "Rhinovirus type 37" and change "Adenovirus" to read "Adenovirus type 5."

- On page 3 of the proposed label, add a description of the composition of surfaces on which the product may be used (e.g., stainless steel, chrome, glass, vinyl) to satisfy DIS/TSS-15 standards.

- On page 3 of the proposed label, add a statement that "Heavily soiled surfaces must be pre-cleaned prior to treatment." Note also that the label no longer includes the following statement, which was previously included on the last-accepted label (dated December 14, 2005): "For all applications, clean surfaces before using the product."

9. The following changes are required on the Technical Bulletin:

- In the footer to all pages, change "Instruction Bulletin" to read "Technical Bulletin."

- In the footer to all pages, add a date reference or version reference so that consumers and regulatory agencies can readily identify whether the Technical Bulletin is the most recent.

- On page 4 of the Technical Bulletin, change "contact intact mucous membranes" to read "contacts intact mucous membranes."

- On page 7 of the Technical Bulletin, re-number the instructions. Currently, there is no #5.

- On page 8 of the Technical Bulletin, change "wood" to "finished wood".

- On pages 9, 10, and 11 of the Technical Bulletin, sections C, D, and G, respectively, include in parentheses the bacteria anf fungi associated with rooted

and unrooted cuttings and cut flowers. As it is currently stated, "bacteria" and "fungi" may include any of the tested bacteria on the label.

- On page 12 of the Technical Bulletin, change "once-though or recycled" to read "once-through or recycled."

- On page 16 of the Technical Bulletin, change "mimimizes turbulence" to read "minimizes turbulence."

- On page 17 of the Technical Bulletin, within the CAUTION text box, change "may not compatible" to read "may not be compatible."

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- On page 18 of the Technical Bulletin, remove cutting boards, as these surfaces are considered porous.

- On page 19 of the Technical Bulletin, delete the word "circulate" or revise the following sentence so that it reads more clearly: "Apply to target surfaces with mop, sponge, circulate, or spray until visibly wet . . . ." The Technical Bulletin for the Selectrocide G-series product line states the following for some applications: "Spray, mop, sponge or swab surfaces or fill, flush, immerse or circulate in tanks, lines, and equipment, ensuring the target surfaces remain visibly wet . . . ."